④ Guelph
April 7, 1979

January 19, 1979

The 4th Plant Development Workshop will be held at the Arboretum Centre, University of Guelph, on Saturday, April 7, 1979.

This note is to invite you and interested graduate students and colleagues to attend. Since the 1979 Annual Meeting of the Canadian Botanical Association is to be held at Carleton University, Ottawa, in June, perhaps there should be less emphasis on formal papers and more time devoted to discussion and posters. With this in mind, we suggest the following tentative format:

1. Contributed papers (restricted to approximately 10 - 15 min. papers in a morning session).
2. A concurrent lunch and discussion around posters.
3. An afternoon discussion session centered around one or more topics. A possible topic might be "Informing undergraduate students about graduate work in Plant Development and graduate opportunities in Canada". Suggestions for other topics are welcome.

Abstracts for papers and posters , and ideas for discussion would be appreciated at an early date, but must be forwarded no later than March 15. Please submit abstracts and discussion ideas to:

Dr. Usher Posluszny
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For further information, do not hesitate to write or phone. Telephone numbers: Usher Posluszny - 519 824-4120, Ext. 2745,
Larry Peterson - 519 824-4120, Ext. 3278.

Sincerely,

Dr. R.L. Peterson,
Larry Peterson

RLP:UP:cp

Dr. U. Posluszny
U. Posluszny

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THE 4th PLANT DEVELOPMENT WORKSHOP

APRIL 7, 1979

AT THE ARBORETUM CENTRE, UNIVERSITY OF GUELPH

Coffee and doughnuts - 9:00 - 10:00 A.M.

Morning Session - 10:00 A.M.

1. Geotonism in plant development
   Roger Horton and Ingrid Boesel - University of Guelph

2. Development and ultrastructure of root nodules of *Eleagnus umbellata*
   William Newcomb - Queen's University

3. The effects of tannic acid and cytochalasin B on microtubule and microfilament distribution in the radish root hair.
   R. W. Seagull and I. B. Heath - York University

4. The generation of variation in nuclear volumes in root meristems
   D. Davidson - McMaster University

5. Histological basis of leaf dimorphism in *Selaginella martensii*
   N. G. Dengler - University of Toronto

6. The structural relationships between mesophyll and bundle sheath cells in *Atriplex* leaves.
   A. A. Krouglicof and R. E. Dengler -- Scarborough College, University of Toronto

Lunch and Poster Session - 12:20 - 2:00 P.M.

Afternoon Session - 2:00 P.M.

7. The growth and development of ophioglossaceous gametophytes under various cultural conditions.
   D. P. Whittier - Vanderbilt University

8. Calcium distribution in globoid crystals of *Cucurbita* cotyledon protein bodies
   John N. A. Lott and Ernest Spitzer - McMaster University

9. Peroxidase synthesis in membranes from cultured peanut cells
   Robert B. van Huystee - University of Western Ontario

10. Organelle specific leucine tRNA and synthetase in soybean (Glycine Max)
    D. Sinclair and D. T. N. Pillay - University of Windsor

Discussion - 3:15 - 4:30 P.M.

Wrap-up - Refreshments - 4:30 - 5:30 P.M.

ABSTRACTS

1. Geotonism in Plant Development

Roger Horton and Ingrid Boesel, Department of Botany and Genetics, University of Guelph.

The effect of gravity on the direction of plant growth - "geotropism" - has been intensively studied for many years. Biologists have also defined other effects of gravity on plants including "geotonic" phenomena where gravity is controlling the rate of intensity of plant growth. Recent investigations have suggested that not only the rate of root growth may be controlled by gravity but that such phenomena as pigment synthesis are also, in part, under geotonic regulation. Using mustard seedlings we have re-examined some of these experimental attempts to establish the reality of geotonic effects in plants and will discuss our results and conclusions.

2. Development and Ultrastructure of root nodules of Elaeagnus umbellata

William Newcomb, Biology Department, Queen's University at Kingston.

Soil actinomycetes, gram variable bacteria exhibiting hyphal growth, form nitrogen fixing root nodules on certain angiosperms including: Myrica, Comptonia, Dryas, Alnus, Sherpherdia and Elaeagnus. The typical nitrogen-fixing endophyte of E. umbellata bears spherical vesicles 2-3μm in diameter. These vesicles have been considered to be the site of the active nitrogenase enzyme but direct evidence for this is lacking. Recently, an actinomycete endophyte from E. umbellata has been isolated and grown in pure culture. When used to inoculate Elaeagnus seedlings in water culture this Frankia sp. isolate causes typical early nodule formation. However, the nodules lack the ability to reduce dinitrogen and the plants die from nitrogen starvation. When these nodules are studied ultrastructurally, the endophyte is shown to lack any vesicles. No other differences are evident between the ineffective nodules and normal effective nodules. Encapsulation of the endophyte is similar in both cases. These observations provide evidence for the hypothesis that the vesicle is the site of active dinitrogen fixation in Elaeagnus nodules.

3. The effects of tannic acid and cytochalasin B on microtubule and microfilament distribution in the radish root hair

R. W. Seagull and I. B. Heath, Department of Biology, York University, Downsview, Ontario, M3J 1P3.

Serial section reconstruction of radish root hairs have produced quantitative descriptions of microtubule (mt) length profiles, which vary along the cell length, and microfilament (mf) densities, which are constant throughout the cell length. Fixation with 0.2% tannic acid has no effect on

mt length profiles, but doubles the number of observable mf in all regions of the hair. Pretreatment with cytochalasin B (CB), at 5 µg/ml, differentially reduces the number of mf at the hair tip, while having little effect in the sub-apical regions. Increased mt length profiles are observed in all regions of CB treated root hairs. These results will be discussed with respect to differential stabilities of cytoskeletal elements in different regions of the hair cell, and the use of CB as an indicator of mf function.

4. The Generation of Variation in Nuclear Volumes in Root Meristems

D. Davidson, Department of Biology, McMaster University, Hamilton.

Nuclear volumes span a wide range of values in many root meristems: a 5 to 8-fold spread of values is common. Volume differences between sister nuclei have been reported, for pairs of nuclei both in sister cells and in binucleate cells. In binucleate cells we have found that the mean ratio of volumes of sister nuclei is 1.17:1 to 1.26:1. In nuclei in sister cells the mean ratio was 1.21:1. Can volume differences of this magnitude generate the observed variability in nuclear volumes in meristems? In Z. mays the mean spread in nuclear volumes was 2.03 ± 0.32-fold in columns of 5-14 cells and 3.1 ± 0.77-fold in columns of 5-19 cells. In V. faba, the mean spread was 1.98 ± 0.61-fold in columns of 6-10 cells and 3.67 ± 1.15-fold in columns of 17-28 cells. It is clear that: 1) a lineage of cells that could be derived from a single initial cell by ∿ 3 mitoses has about a 2-fold spread of nuclear volumes; 2) once ∿ 4 divisions have been completed there is always about a 3-fold spread. The differences between volumes of sister nuclei parallel data reported for wheat roots that cytoplasmic division at mitosis is unequal. The joint contributions of cytoplasmic and nuclear inequalities arising at mitosis are sufficient to generate the observed variation in nuclear and cell size in root meristems.

5. Histological basis of leaf dimorphism in Selaginella martensii

N. G. Dengler, Department of Botany, University of Toronto, Toronto, Ontario.

The dorsal and ventral leaves of the plagiotropic shoots of Selaginella martensii show a strong dimorphism in morphology and size brought about by differences in the number, sizes and shapes of individual cell types. The difference in area between the dorsal and ventral leaves is correlated with the number of epidermal cells per layer, indicating that cell division is the primary developmental mechanism resulting in anisophylly. Leaf thickness in the ventral leaves is 1.5 x that of the dorsal leaves, reflecting a greater number of mesophyll cells and more intercellular space per unit area. Dimorphism is also expressed in the shape of the major photosynthetic cells which are those of the adaxial epidermis of the ventral leaves and the abaxial epidermis in the dorsal leaves. Stomata do not show the same developmental flexibility and always differentiate on the abaxial leaf surface. This description of mature leaf structure provides the basis for understanding the developmental mechanisms resulting in leaf dimorphism.

6. The structural relationships between mesophyll and bundle sheath cells in Atriplex leaves

A. A. Krouglicof and R. E. Dengler, Life Sciences Division, Scarborough College, University of Toronto, West Hill, Ontario.

The quantitative leaf anatomy and plasmodesmatal frequencies were compared in one C-4 species, Atriplex rosea, and two C-3 species, A. hortensis and A. hastata. Results concerning anatomical parameters were obtained using 2 µm thick sections and leaf clearing. A. rosea (C-4) was found to have a higher percentage of bundle sheath-mesophyll cell contact, percentage of total cell contact, and percentage volume of bundle sheath cells per volume of leaf, as well as more vein length per leaf surface area. The ratio of inner exposed surface area to outer exposed surface area appears to be correlated with leaf thickness rather than with the type of carbon fixation pathway in the species examined. Fine structure analysis of three wall interfaces (bundle sheath-mesophyll, mesophyll-mesophyll, and bundle sheath-bundle sheath) showed no significant differences in the internal structure of the plasmodesmata or in the wall and cytoplasm adjacent to them. However, the distribution and frequency of these intercellular connections differed. In the mesophyll-bundle sheath junction of the C-4 Atriplex species, the number of plasmodesmata per unit area were found to be over ten times higher than in the other species and in the other wall interfaces of the same species. Plasmodesmata were counted using micrographs of transversely sectioned cell walls, taking into account the thickness of the section and the radii of the plasmodesmata (Robards, 1976).

7. The growth and development of ophioglossaceous gametophytes under various cultural conditions

D. P. Whittier, Biology, Vanderbilt University, Nashville, Tennessee.

Spores of Botrychium spp. and Ophioglossum spp. were sown on a nutrient medium containing minerals, 0.6% agar and usually 0.5% sucrose. Of the 5 species undergoing germination, the time for germination in the dark varied from 3 weeks to 6 months. Ophioglossum engelmannii appears to require a cold treatment before germination can take place. None of the spores germinated in the light. A carbon source is necessary for the growth of these gametophytes beyond the 2-3 celled stage. Growth of Botrychium spores on sterilized soil ceased after 2-3 cells supporting the concept that the endophytic fungus supplies the gametophyte with organic nutrients in nature. A variety of sugars can act as the carbon source but insoluble carbon sources fail to support the growth of these gametophytes. Spores germinated on media with an appropriate carbon source produced normal mature gametophytes with sex organs. None of these gametophytes from axenic culture contained the endophytic fungus found in the gametophytes from nature.

8. Calcium Distribution in Globoid Crystals of Cucurbita Cotyledon Protein Bodies

John N.A. Lott and Ernest Spitzer, Department of Biology, McMaster University.

Energy dispersive x-ray analysis was used to investigate the location of globoid crystals with relatively high calcium levels within cotyledons of Cucurbita maxima, Cucurbita mixta and Cucurbita andreana. The small globoid crystals in both upper and lower epidermal cells commonly contained calcium. Calcium was present in globoid crystals of all pro-vascular regions with the exception of the very small provascular regions of C. maxima. In C. maxima and C. mixta cotyledons, some cases were observed where calcium was found in the globoid crystals of the first layer of mesophyll cells surrounding the provascular region, but in general, calcium was absent from globoid crystals of palisade and spongy mesophyll cells. In C. andreana, globoid crystals of palisade and spongy mesophyll cells commonly contained at least some calcium. Cell position and cell type are factors affecting the calcium content of globoid crystals in protein bodies.

9. Peroxidase Synthesis in and Membranes from Cultured Peanut Cells

Robert B. van Huystee, Department of Plant Sciences, University of Western Ontario, London, Ontario.

Peroxidase synthesis in peanut cells grown in suspension culture was only affected by incubations with actinomycin D during a brief time following the lag phase of growth. A greater effect was noted at that time using cycloheximide, with lesser effects at later phases. Incubation with chloramphenicol had no effect. These observations along with the presence of peroxidase linked to the endoplasmic reticulum, suggest synthesis of the enzyme occurs on these organelles.

10. Organelle Specific Leucine tRNA and Synthetase in Soybean (Glycine Max)

D. Sinclair and D. T. N. Pillay, Department of Biology, University of Windsor, Windsor, Ontario

Organelle specific leucine transfer RNA and synthetase were isolated and some of their properties studied. Hydroxylapatite column chromatography of leucyl-tRNA synthetase from the whole soybean cotyledon resolved into three isozyme peaks of the enzyme. However, when the synthetase was extracted from specific organelles and subsequently acylated with either homologous or heterologous tRNA, each of the isozyme peaks was found to be localized in different cell compartments (viz: cytoplasm, mitochondria and chloroplasts).

Reverse-phase chromatography (RPC-5) of the ^{3}H-leucyl-tRNA showed six isoaccepting peaks for the whole cotyledons and the organelle specific tRNA synthetase was found to acylate specific isoacceptors on the basis of their chromatographic behaviour on RPC-5 columns.

POSTER

11. The Recognition of Proteins Modified by 2,4-D During Plant Tissue Culture

R. Waern

Isoelectric focussing followed by SDS/glycine discontinuous acrylamide gel electrophoresis are being used in an attempt to recognize proteins that have been modified, either quantitatively or qualitatively, by the presence of 2,4-D in plant tissue culture media during culture of white Navy bean hypocotyls.

The detection of quantitatively modified proteins would support the proposed hormonal role of 2,4-D, perhaps as an inducer molecule during transcription of RNA, while qualitative differences might suggest mutagenic capabilities of the herbicide.

Both types of proteins have been tentatively identified in the gels.

POSTER

12. Structure of the meristem and cap of Ephedra roots

R. L. Peterson and Janet Vermeer, Department of Botany and Genetics, University of Guelph, Guelph, Ontario.

Ephedra, a gymnosperm, has a root with an extensive root cap. Divisions continue in the columella for a considerable distance, distal to the apical meristem. Lower columella cells synthesize large starch grains which become distally displaced. Outer peripheral root cap cells which have a common origin with the cortex, have less starch and become very vacuolated. Microspectrophotometric determinations of DNA levels indicate that cells of the columella and periphery remain at either the 2C or 4C level. The apical meristem consists of a group of cells which have been referred to as "root initials" in the literature. These cells, however, do not incorporate ^{3}H-thymidine over a 24 hour period whereas many nuclei of adjacent cells become labelled. These "root initials" form part of the quiescent centre of the root meristem.